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Associations Between Female Reproductive Traits and Polychlorinated Biphenyl Sediment Concentrations in Wild Populations of Brown Bullhead (*Ameiurus nebulosus*)

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Abstract Aquatic contaminants, specifically polychlorinated biphenyls (PCBs), a class of persistent organic contaminants, have been associated with sublethal effects on reproduction in fishes. Female brown bullhead (*Ameiurus nebulosus*) were used to assess variation in reproductive traits across eight populations differing in sediment sum PCB concentrations in the Lower Great Lakes region. Differences in maternal carotenoid allocation patterns among these populations were also examined. No significant associations were found between sediment sum PCB concentrations corrected for organic content (OC) and reproductive traits. However, egg diameter was negatively correlated with sediment PCB concentrations not corrected for OC, suggesting that observed relationships between sediment sum PCB concentrations and reproductive traits are driven by classes of environmental contaminants whose bioavailability are not predicted by OC, such as metals. An unexpected positive relationship was also found between egg carotenoid concentrations and sediment PCB concentrations. This positive relationship was explained by the maternal allocation of carotenoids based on a negative correlation between female muscle and egg carotenoid concentrations, where females from less contaminated locations had lower egg and greater muscle carotenoid concentrations than those from more contaminated locations. The results of this study identify sublethal effects of environmental contaminants on reproductive life-history traits in female brown bullhead, and investigations of adaptive mechanisms underlying this variation are warranted.

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Aquatic contaminants have been associated with numerous sublethal effects on reproduction in female fish (e.g., see reviews in Kime 1995; Kime and Nash 1999; Islam and Tanaka 2004; Hewitt et al. 2008). It is important to identify these associations because many female reproductive traits, including egg quality, play significant roles in determining offspring fitness (Brooks et al. 1997; Bobe and Labbé 2010). In wild fish populations, exposure to contaminants has been linked to endocrine disruption and decreases in gonadosomatic index (GSI) in yellow perch (*Perca flavescens*) (Hontela et al. 1995) and snakehead (*Channa punctatus*) (Bhattacharya 1993), decreases in fecundity in white croaker (Hose et al. 1989), decreases in egg diameter and fecundity in white sucker (*Catostomus commersoni*) (Munkittrick and Dixon 1988), and delays in egg development in fathead minnow (*Pimephales promelas*) (Kidd et al. 2007). These changes in female reproductive traits have been shown to reflect the relative degree of environmental contamination to which an individual is exposed with improvement in reproductive traits in locations where contaminant exposure was mitigated (Munkittrick et al. 1994; Farwell et al. 2012). The plasticity of female reproductive traits and their importance for offspring survival has made those traits common candidates for biomonitoring programs focusing on the effects of environmental contaminants on reproduction and population dynamics.

The chemical properties of eggs that reflect egg quality can also be affected by environmental contaminants. One class of compounds that has the potential to be used as an indicator of contaminant exposure is carotenoids. Carotenoids are synthesized in plants and some bacterial and fungi

(Olsen and Owens 1998). They are assumed to be a limiting resource in most animal species because they cannot be synthesized de novo (Olsen and Owens 1998) and therefore are consumed by animals and distributed to most tissue types. Maternal deposition results in egg yolks also containing carotenoids, causing their red to yellow colouration (Surai and Speake 1998; Blount et al. 2000). The amount of carotenoids deposited into the egg has generally been found to be positively related to the amount of carotenoids the mother possesses (Blount et al. 2002; McGraw et al. 2005; Grether et al. 2008). Carotenoid concentrations have been associated with increases in immune function and have antioxidant properties in both adults (Krinsky 2001; Blount et al. 2002) and embryos (Tyndale et al. 2008). Increased egg carotenoid concentrations have been linked to decreases in oxidative damage and neural development deformities (Surai and Speake 1998) and increased immune responses in embryos (Sanio et al. 2002), presumably due to increased antioxidant function, and ultimately have implications for offspring fitness (Tyndale et al. 2008). Metabolism of environmental contaminants, by both adults and embryos, can increase the amount of reactive oxygen species produced, potentially increasing oxidative stress (Di Giulio et al. 1989). Despite this connection, little research has focused on identifying relationships between environmental contaminants and egg carotenoid concentrations as they relate to offspring fitness in wild populations, with none being performed in fish. Møller et al. (2005) found lower carotenoid concentrations in blood, liver, and eggs from wild female barn swallows (*Hirundo rustica*) exposed to radiation contamination compared with those from non-contaminated locations. This relationship was explained by a greater proportion of carotenoids in the contaminated birds being sequestered by the greater concentration of free radicals produced by radiation. This resulted in a lower proportion of available carotenoids to birds exposed to radiation compared with those not exposed to radiation. Similarly, adult birds living in more polluted urban environments had relatively lower liver carotenoid concentrations than those from rural environments (Møller et al. 2010).

In ecotoxicological studies performed using wild organisms, average values of traits of interest are often compared among populations originating from locations that are expected to differ in their relative levels of environmental contaminants. It can also be beneficial to identify specific and biologically relevant contaminants to more rigorously compare the locations and assess the ecological implications of the contaminants. This notion assumes that measured contaminants are mechanistically linked to measured biological effects. One class of contaminants that has been linked to decreases in reproductive trait development in fish is polychlorinated biphenyls (PCBs) (Hewitt & Servos 2001). In a laboratory study, oral doses of PCBs resulted in significant decreases in fecundity and offspring survival in minnows (*Phoxinus phoxinus*) (Bengtsson 1980). Similar results were found in a wild population of Chinook salmon (*Oncorhynchus tshawytscha*) from Lake Michigan where increases in sum PCB concentrations in muscle tissue were associated with decreases in fecundity and hatching success (Ankley et al. 1991). These differences in reproductive traits have been suggested to be due to changes in metabolic function (Beyers et al. 1999), toxic properties of planar PCB congeners on liver functioning and subsequent vitellogenin production (Kime 1995; Johnson et al. 1997), and changes to egg structure, including oocyte walls (Kime 1995). PCBs are also known to be correlated with several other organic contaminants in natural habitats (Szalinska et al. 2011) and are therefore a good metric to assess general environmental contaminant gradients.

Abundant and native to North America, brown bullhead (*Ameiurus nebulosus*) are tolerant of a variety of environmental stressors, including contaminants (e.g., Pyron et al. 2001). They are a philopatric species and therefore are likely to be exposed to contaminants from a localized region compared with species with larger home ranges. A study by Sakaris and Jesien (2005) using ultrasonic telemetry showed the average home range of an adult brown bullhead to be 500 m during reproduction and $\leq$3.1 km during the fall when the fish are retreating to deeper waters to overwinter. Brown bullhead are also benthic throughout the majority of their life and are therefore exposed to contaminants that accumulate in sediments. The majority of research on brown bullhead has focused on their tumour formation when exposed to environmental carcinogens, so much so that they are used by the International Joint Commission as an indicator species for contaminated habitats (IJC 1989). Despite this amount of research on brown bullhead contaminants, little is known about the effects of contaminants on their reproduction and life histories (however, see Lesko et al. 1996).

Brown bullhead were used to examine whether associations exist between female reproductive traits and sediment sum PCB concentrations found in the Huron–Erie Corridor in the Laurentian Great Lakes. A recent study by Farwell et al. (2012), performed in the Detroit River region of the Huron–Erie Corridor, examined the acclimation of female brown bullhead reproductive life-history traits by experimentally manipulating environmental contaminant exposure. They found that females collected from contaminated locations that were subsequently allowed to clear their contaminants over a period of 1 year in seminatural experimental ponds resulted in lower egg sum PCB concentrations, as well as greater egg diameters and GSI, compared with those examined directly after collection from the same contaminated locations. In the current study, we assessed whether similar relationships between sediment sum PCB concentrations and reproductive traits are present across a broader range of wild populations in terms of both geography and relative contaminant concentration. Sediment samples, rather than individual tissues, were used to determine contaminant exposure and whether sediment samples may be useful for relatively rapid habitat assessment compared with lethal sampling of fish. It was predicted that females from locations with relatively lower sediment sum PCB concentrations would have larger egg diameters and greater fecundities, GSIs, and egg carotenoid concentrations than females from locations with relatively greater sediment sum PCB concentrations.

Methods

Sample Collection

Gravid female brown bullhead were collected using fyke nets and boat electroshocking from May 10 to June 8, 2010, from eight locations in the Lower Great Lakes region: Belle River (BR; 42°17′39″N, 82°42′43″W), Turkey Creek (TU; 42°14′43″N, 83°6′19″W), Fox Creek (FC; 41°59′48″N, 82°50′56″W), Puce River (PR; 42°18′8″N, 82°46′50″W), Peche Island (PI; 42°20′38″N, 82°55′43″W), Cedar Creek (CC; 42°0′42″N, 82°47′13″W), Trenton Channel (TC; 42°10′33″N, 83°9′16″W), and Belle Island (BI; 42°20′57″N, 82°58′31″W) (Fig. 1). These locations have been the focus of ecotoxicological studies (e.g., Farwell et al. 2012; Leadly et al. 1999). Of these locations, PI, TC, and BI are included in the Detroit River Area of Concern (IJC 1978); CC is located within a heavily used marina; and PR runs through a residential area. All locations have been showed to vary in environmental contaminants (Drouillard et al. 2006) likely due to different contaminant source inputs and hydrological activity.

Sediment samples were collected from each of the eight locations to assess sum PCB concentration in the environment. Three sediment samples of ~20 g of each were obtained from the sediment surface of each location using a petite ponar. Each sediment sample was taken within 5 m of the fish collection site. The sediment samples were combined and homogenized to produce a single sample for each location and stored at −20 °C before PCB analyses (Ali et al. 1993). Each sediment sample was taken at ~1-m water depth, except at BI where they were taken at 2-m water depth. All sediment samples were similar in type (silt) and contained small amounts of organic debris, which were removed before analysis.

Life-History Trait Assessment

Female bullheads were transported immediately after capture to a research facility at Leadley Environmental Ltd. (42°6′14″N, 82°55′47″W) and killed using a lethal dose of MS-222. All egg samples for analysis were obtained and stored within 5 min after killing. Total length and mass measurements were taken and used to calculate Fulton's condition factor (C = body mass/(total length)^3 [Ricker 1975]) because PCB accumulation and egg development can depend on body condition (Brooks et al. 1997). Ovaries were then removed, patted dry with paper towel, and their mass (g) recorded to estimate gonadosomatic index [GSI = ovary mass/(total mass − ovary mass)]. A subsample of eggs of known mass was taken to estimate fecundity; 50 were individually measured and used to calculate average egg diameter (mm). The remaining eggs were divided into subsamples of a known mass, placed in 1.5-mL Fisherbrand Cryovials (Thermo Scientific, Waltham, MA, USA), and snap frozen in liquid nitrogen. Samples for carotenoid analysis were stored at −80 °C, and samples for lipid analysis were stored at −20 °C. Approximately 2 g of dorsal muscle tissue was excised and stored at −80 °C for carotenoid analysis. A pectoral spine was removed for age determination according to the methods in Blouin and Hall (1990), and visible annual rings were counted under a zoom stereomicroscope (SZX7 Olympus; www.olympusamerica.com).

Sediment PCB Extraction and Analyses

Sum PCB sediment concentration was determined from 20 g of sediment homogenate according to the protocol outlined by Drouillard et al. (2006). Sediment samples were ground with 100 g of anhydrous sodium sulphate (ACS grade, BDH, Ontario, Canada) to remove moisture, and Soxhlet extractions for organic contaminants were performed using 300 mL of acetone and hexane solution (1:1 ratio) for 24 h. Five samples were run concurrently, including a method blank to estimate percent recovery and an in-house dry sediment reference sample (National Institute of Standards and Technology—Standard Reference Material 1944) to estimate interassay variation. All samples were spiked with an internal recovery standard of 200 ng_{PCB30}/mL. After extraction, the extract was then condensed to approximately 2 mL using a rotary evaporator. The extracts were then back-extracted using a separatory funnel containing water and hexane over three solvent washings. Hexane from each washing was combined and dried over sodium sulphate. The extract was condensed again to ~2 mL, and florisil chromatography was used for sample cleanup. Fraction 1 was eluted with 50 mL of hexane and fraction 2 with 50 mL of dicloromethane:hexane (15:85 ratio). Fractions were condensed again, and 0.2–0.5 g of activated copper was added to remove sulphur. Analysis of fractions was performed using a Hewlett Packard 5890 gas chromatograph (Avondale, Pennsylvania, USA) with an electron capture detector. The following PCB

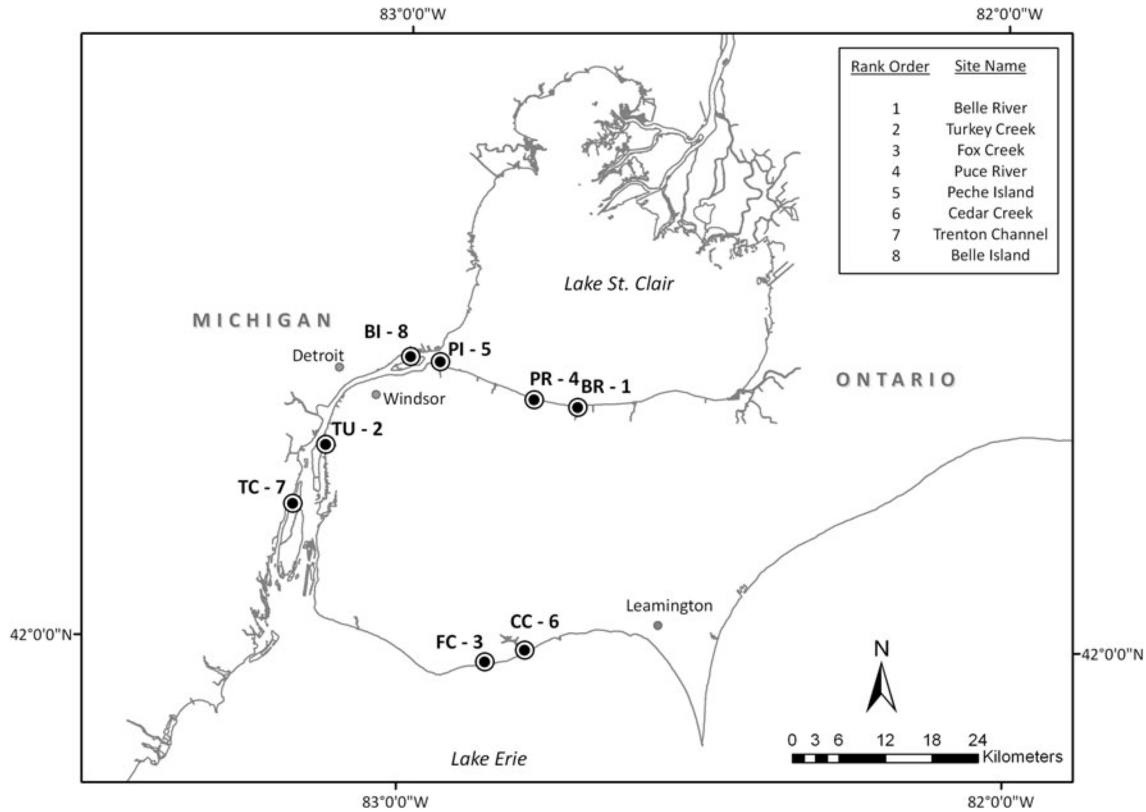

Fig. 1 Map of the eight female brown bullhead (*Ameiurus nebulosus*) collection locations in the Lower Great Lakes region. Locations are labelled in rank order from lowest (*1*) to greatest (*8*) sediment sum PCB concentration (dry weight)

congeners (IUPAC numbers, coeluting congeners separated by slash) were identified by retention time and referenced against an external PCB standard (Quebec Ministry of Environment Congener Mix; AccuStandard, New Haven, Connecticut, USA): 18, 17, 28/31, 33, 52, 49, 44, 74, 70, 95, 101, 99, 87, 110, 82, 151, 149, 118, 153/132, 105, 138, 158, 187, 183, 128, 177, 171, 156, 180, 170, 199, 208, 195, 194, 205, 206, and 209). Sum PCB concentrations (dry weight [dw]) were calculated for each sediment sample as the sum of concentrations of each of the above-mentioned congeners. Mean percent recovery of the PCB34 spike was 99.96 ± 6.74 % (mean ± SD), and thus sample concentrations were not recovery-corrected. All sum PCB in-house homogenate samples were in compliance (mean ± 2 SD) with the Great Lakes Institute for Environmental Research analytical laboratory quality-assurance guidelines (Canadian Association for Environmental Analytical Laboratories Accreditation) and were ISO17025 certified.

Total Organic Content Analyses

The bioavailability of PCBs in sediment, and other nonpolar organic contaminants, is dependent on the organic content (OC) of the sediment (Landrum and Faust 1994); therefore, sediment PCB concentrations should be OC-corrected when comparing tham with reproductive traits. OC was determined using the loss-on-ignition procedure and using a Caarlo Erba Elemental Analyzer for total organic carbon (Drouillard et al. 2006). Preweighed sediment samples were combusted at 450 °C for 24 h, and the total organic carbon content was then determined gravimetrically.

Nevertheless, associations between uncorrected sediment PCB concentrations and reproductive traits are still of value before the correction because they can indicate potential relationships with other inorganic contaminants, such as heavy metals. We therefore examined relationships between sum PCB concentrations and reproductive traits using dry and OC-corrected sediment samples.

Carotenoid Extraction and Analyses

According to Li et al. (2005), with minor modifications, carotenoid extractions from 1 g of egg (70–100 eggs) or muscle tissue were performed under dim light and on ice. Thawed egg or muscle samples were homogenized and extracted in 2 mL of acetone. The mixture was then centrifuged at 3,500 rpm for 5 min at 4 °C and the supernatant removed. This was repeated two more times with 1 mL of acetone. Supernatants were pooled, and 1 mL each of MtBE, hexane, and distilled water was added and centrifuged at

3,500 rpm for 5 min at 4 °C to facilitate phase separation. The organic supernatant containing carotenoids was pipetted out and dispensed into a glass vial. Phase separation was repeated three times. Supernatants were homogenized, evaporated, and sealed under nitrogen gas.

High-performance liquid chromatography was used to characterize egg carotenoids from 16 individuals across a subset of 4 locations (PI, BR, BI, and TC) using a Waters 2695 HPLC coupled to a Waters 487 dual-channel ultraviolet (UV)-visible detector (according to Chui et al. 2001). Samples were suspended in 200 µL of mobile phase containing a mixture of methanol, acetonitrile, and dichloromethane (42:42:16, v/v/v) and filtered through a Teflon membrane filter (0.45-µm) to remove particulates. A gradient elution of the same mobile phase concentration was used over 30 min, and carotenoids were detected and quantified with an absorbance response at a maximum wavelength of 480 nm. Carotenoids were identified by comparison with responses of known carotenoid standards. Lutein and zeaxanthin (xanthophyll carotenoids) combined constituted, on average, 80 % ± 4.99 (mean ± SD) of all carotenoids in each egg sample (PI = 81.75 % ± 4.79′ BR = 82.0 % ± 6.27; BI = 77.75 % ± 5.06; and TC = 79.5 % ± 3.87). All other carotenoids present were also identified as xanthophyll carotenoids and were present in relatively small and consistent amounts across the samples. Because specific carotenoid identification was not required to assess the entire carotenoid concentration of each sample, a generalized method for quantifying total xanthophyll concentration using spectrophotometry was followed for all egg and muscle samples as described later in the text.

Total xanthophyll carotenoid concentrations were measured using a Beckman DU50 UV–visible spectrophotometer (McGraw et al. 2001). Each sample was individually suspended in 1 mL of ethanol, and absorbance was measured at a wavelength of 448 nm (λ_{max} for xanthophylls). Quantification of carotenoid concentration in both egg and muscle tissues (µg carotenoid/g of tissue) was determined using the following equation: [(A × volume of extract (mL of))/(E × sample mass (g))] (McGraw et al. 2001) where A is the absorbance of the sample, and E is the extinction coefficient at 1 %/1 cm of the relevant carotenoids at λ_{max} (2,550 for xanthophylls in ethanol). Each sample extraction was analyzed on the spectrophotometer in triplicate with an average variation of 0.53 %. Replicate extractions of 14 samples (7 from BR and 7 from TC) were also performed to estimate extraction efficiency with an average variation of 1.3 %.

Lipid Extraction and Analyses

Percent neutral lipids in eggs were measured because this has been linked to egg quality, carotenoid concentration, and concentration of lipophilic contaminants (Goodwin 1980; Brooks et al. 1997; Webster et al. 1999). Percent neutral lipids were determined gravimetrically for ~0.5 g of egg tissue according to the dichloromethane (DCM)/hexane extraction procedure described in Drouillard et al. (2004). Briefly, egg tissue was ground with 15 g of activated sodium sulphate and added to 20-mL glass syringes containing 15 mL of DCM and hexane (1:1); each was connected to 1-µm glass fibre syringe filter and fitted to a solid-phase extraction manifold (Phenomenex). Solvent extracts were collected and roto-evaporated to approximately 2 mL. Solvents were then reconstituted in hexane to 10 mL from which 1 mL of samples were placed in preweighed aluminum weigh boats. Boats containing extracts were evaporated at room temperature and dried for 1 h at 100 °C. Boats were then weighed and percent total lipid calculated using the following equation: [((boat mass after drying − boat mass before drying)/sample mass) × 1000].

Statistical Analyses

Linear regression with multiple values of *Y* for each value of *X* (Sokal and Rohlf 1995, p 476) was used to examine variation in egg diameter (log-transformed), fecundity, GSI, and egg carotenoid concentration in relation to the single sum PCB sediment concentration value obtained for each location. This analysis was used because it is able to separate out the deviation from regression sums of squares and provide an independent estimate of error among *Y* values for each value of *X* compared with regressions with a single value of *Y* for each value of *X* where there is only an unexplained sum of squares (Sokal and Rohlf 1995). Normality was tested for each variable using Shapiro–Wilk goodness-of-fit test, and homogeneity of variance was tested for each variable using Levene's test.

Results

Sediment sum PCB concentrations across the eight locations ranged from 7.18 to 33.45 µg/kg dry weight (Table 1). There was no significant difference in percent lipid concentration of eggs ($F_{1,65} = 1.28$, $P = 0.27$) or Fulton's condition factor of females ($F_{1,65} = 0.78$, $P = 0.60$) across locations. There was no difference in mean fish age between locations with the exception of Fox Creek where one female was 9 years of age (Table 1).

Using sediment sum PCB concentrations corrected for OC on a dry-weight basis, there were no significant relationships between sediment sum PCB concentration and egg diameter ($r^2 = 0.16$, $P = 0.14$), fecundity ($r^2 = 0.02$, $P = 0.86$), GSI ($r^2 = 0.14$, $P = 0.18$), or egg carotentoid concentration ($r^2 = 0.04$, $P = 0.98$).

Table 1 Means ± SD (range) and sample sizes (*n*) for life-history and egg quality metrics of female brown bullhead (*Ameiurus nebulosus*) collected from eight locations in the Huron–Erie corridor

Location	*n*	Sum PCB (µg/kg dw)	OC (%)	Total length (mm)	Total mass (g)	Condition (mass/TL^3)	Age (years)	Egg diameter (mm)	Fecundity (# eggs)	GSI (%)	Carotenoid concentration (µg/g egg)	Carotenoid concentration (µg/g muscle)	Total lipids (% egg)
Belle River	9	7.18	3.46	313 ± 24.1 (217–425)	393.9 ± 35.2 (321–657)	1.94 ± 0.8 (0.86–3.14)	4 ± 0.3 (2–5)	2.51 ± 0.02 (2.4–2.59)	5677 ± 442 (5030–6390)	11.32 ± 0.89 (9.82–12.55)	2.07 ± 0.15 (1.91–2.33)	0.56 ± 0.02 (0.46–0.60)	6.51 ± 0.24 (5.67–7.75)
Turkey Creek	7	8.96	4.07	236 ± 7.8 (203–266)	295.8 ± 11.2 (281–369)	2.03 ± 0.7 (1.96–3.36)	4 ± 0.2 (3–4)	2.56 ± 0.01 (2.51–2.6)	5723 ± 450 (4898–6200)	12.41 ± 1.4 (10.54–14.73)	2.00 ± 0.10 (1.86–2.14)	0.61 ± 0.01 (0.57–0.65)	6.85 ± 0.21 (5.98–7.53)
Fox Creek	6	10.75	2.40	359 ± 34.6 (256–462)	420.4 ± 43.3 (366–634)	1.95 ± 0.9 (0.64–2.18)	6 ± 1 (3–9)	2.48 ± 0.03 (2.41–2.57)	6047 ± 556 (5299–6753)	11.38 ± 0.97 (10.24–12.61)	2.03 ± 0.11 (1.82–2.13)	0.59 ± 0.01 (0.56–0.62)	7.06 ± 0.57 (4.36–8.12)
Puce River	9	17.09	3.64	276 ± 13.9 (212–351)	357.5 ± 17.8 (294–483)	1.56 ± 0.9 (1.17–3.08)	4 ± 0.3 (3–5)	2.49 ± 0.02 (2.41–2.56)	5299 ± 385 (4645–5720)	10.34 ± 0.95 (9.21–11.75)	2.22 ± 0.29 (1.96–2.87)	0.54 ± 0.01 (0.47–0.59)	6.45 ± 0.36 (4.95–8.75)
Peche Island	8	17.64	14.71	282 ± 23.1 (197–397)	350.2 ± 27.9 (296–502)	1.62 ± 0.7 (0.80–3.87)	4 ± 0.2 (3–5)	2.58 ± 0.01 (2.51–2.6)	5866 ± 341 (5380–6390)	13.08 ± 1.6 (10.31–15.22)	2.05 ± 0.10 (1.90–2.16)	0.59 ± 0.02 (0.49–0.66)	6.73 ± 0.30 (5.39–8.01)
Cedar Creek	10	22.16	9.33	292 ± 20.7 (216–387)	353.4 ± 30.5 (268–543)	1.63 ± 0.6 (0.94–2.66)	4 ± 0.4 (3–6)	2.40 ± 0.01 (2.34–2.57)	5646 ± 300 (5287–6753)	10.70 ± 0.90 (9.12–11.88)	2.89 ± 0.21 (2.12–3.13)	0.52 ± 0.01 (0.46–0.58)	7.61 ± 0.32 (5.55–9.01)
Trenton Channel	11	28.71	9.87	266 ± 12.0 (201–322)	313.2 ± 13.2 (267–421)	1.65 ± 0.7 (1.26–3.29)	4 ± 0.2 (3–5)	2.40 ± 0.02 (2.33–2.5)	5225 ± 519 (4338–6001)	8.92 ± 1.4 (7.18–11.00)	2.86 ± 0.34 (2.99–4.30)	0.46 ± 0.01 (0.39–0.56)	6.97 ± 0.39 (4.69–8.73)
Belle Island	6	33.45	3.97	292 ± 24.6 (192–369)	333.8 ± 20.3 (294–451)	1.25 ± 0.6 (0.90–4.15)	3 ± 0.3 (3–5)	2.42 ± 0.01 (2.38–2.46)	5520 ± 460 (5190–6412)	9.73 ± 0.97 (8.25–11.30)	2.86 ± 0.24 (2.55–3.11)	0.48 ± 0.01 (0.44–0.51)	7.17 ± 0.27 (6.41–8.11)

Sum PCB concentration (dry weight) of homogenized sediment samples is provided as a single measurement for each location along with organic content for each location (%) (see "Methods" section for details)

Using sediment sum PCB concentrations not corrected for OC on a dry-weight basis resulted in a significant negative relationship between sediment sum PCB concentration and egg diameter ($r^2 = 0.39$, $P = 0.035$; Fig. 2a). There was no significant relationship between sediment sum PCB concentration and fecundity ($r^2 = 0.11$, $P = 0.12$; Fig. 2b), or GSI ($r^2 = 0.29$, $P = 0.073$; Fig. 2c). There was a significant positive relationship between sediment sum PCB concentration and egg carotenoid concentration ($r^2 = 0.62$, $P = 0.01$; Fig. 2d). To assess whether these observed relationships resulted from covariance between sum PCB and metal concentrations (inorganic contaminants), because PCB concentrations were not corrected for OC, principal component analysis (PCA; verimax orthogonal rotation), including historical metal and sum PCB concentrations obtained from a Detroit River area sediment-monitoring program implemented in 1999 (described by Drouillard et al. 2006), was performed. From this historical database, data had only been reported for four of our eight study locations: BI, PI, TU, and TC. This analysis showed two PCs that combined explain 86 % of the variation in the dataset. The most descriptive axis, PC1, explained 62 % of the variation and showed loadings ranging from 0.62 to 0.79 for 11 of the 19 metals as well as dry-weight sediment sum PCB concentrations. The metals with the greatest loadings (>0.7) included copper, iron, nickel, and lead. PCA was not performed for carotenoid concentrations because no historical data exist.

The positive relationship between egg carotenoid concentration and sediment sum PCB concentration not corrected for OC was unexpected, and a post hoc comparison between egg and muscle carotenoid concentration was performed to investigate trends in maternal allocation of carotenoids. A significant negative relationship was found between egg and muscle carotenoid concentrations ($r = -0.91$, $P = 0.0018$), with females from less contaminated environments (sediment sum PCB concentrations) having lower egg carotenoid concentrations and greater muscle carotenoid concentrations compared with females from more contaminated locations (Fig. 3).

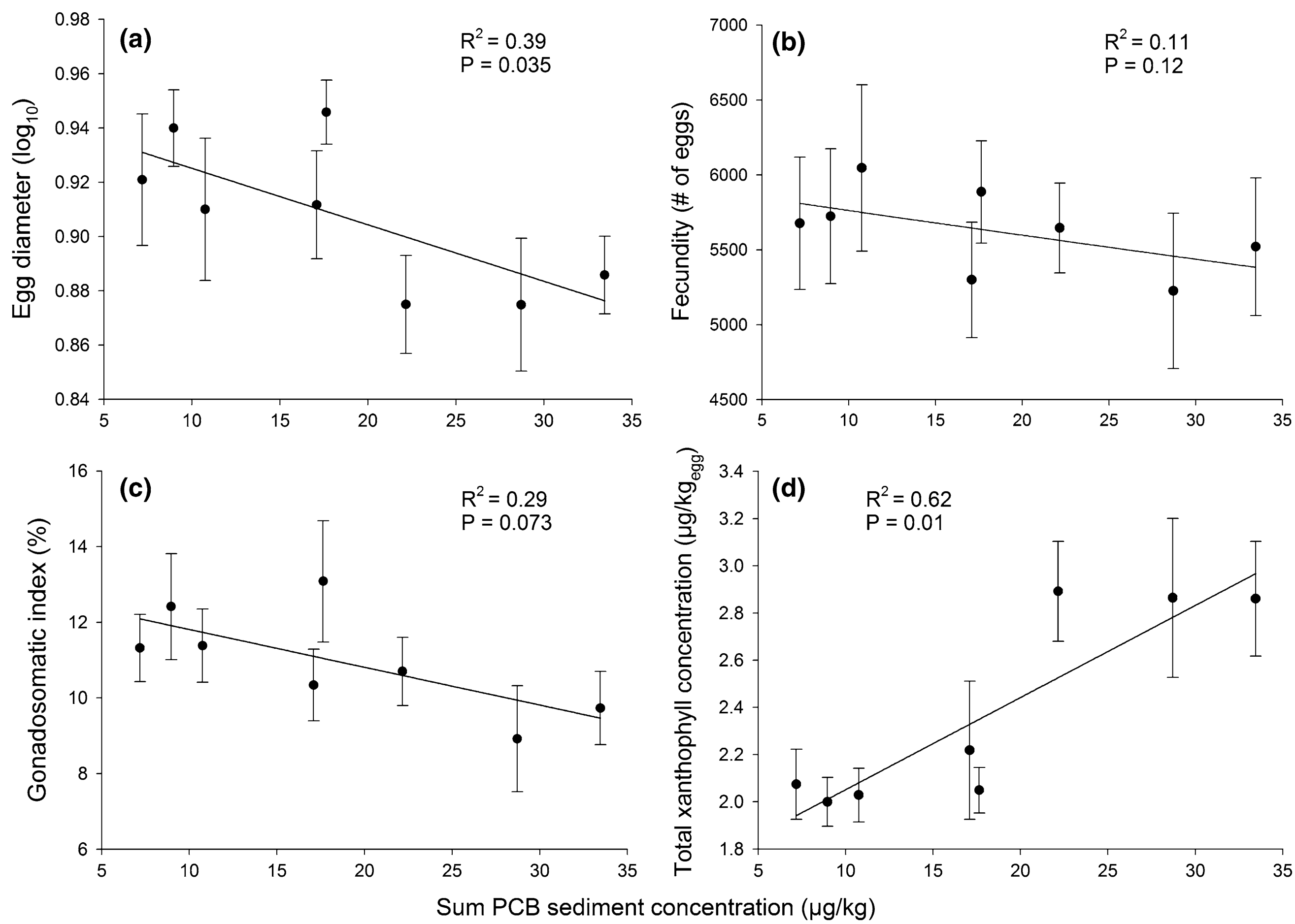

Fig. 2 Relationships between sediment sum PCB concentrations (dry weight) from the eight locations and **a** mean egg diameter ($\log_{10}$), **b** mean fecundity (no. of eggs), **c** mean GSI (%), and **d** mean egg total xanthophyll carotenoids (µg/kg) in female brown bullhead (*A. nebulosus*). *Error bars* = 1 SD

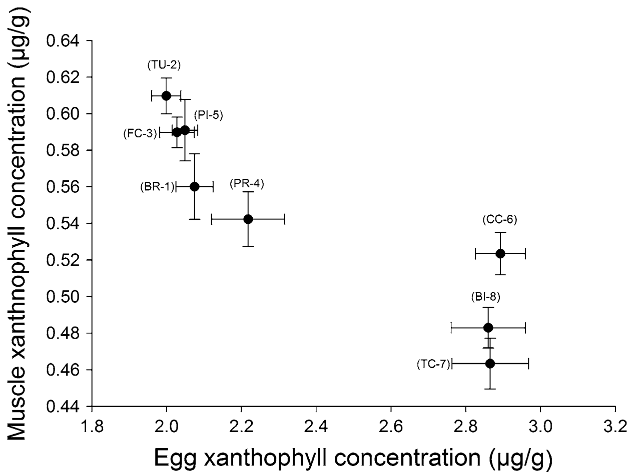

Fig. 3 Relationship between mean muscle xanthophyll carotenoid concentration (μg/g) (± SD) and mean egg xanthophyll carotenoid concentration (μg/g) (±1 SD) for each location. Locations are labelled in rank order from lowest (*1*) to greatest (*8*) sediment sum PCB concentration (dry weight) corresponding to Fig. 1

Discussion

Female brown bullhead reproductive traits are not affected by sum PCB concentrations in sediment corrected for OC from different locations in the Lower Great Lakes region. When using sum PCB concentrations in sediment not corrected for OC, negative relationships were identified with egg diameter and GSI; only that of egg diameter was statistically significant. There was also a significant positive relationship between egg carotenoid concentration and sediment sum PCB concentrations, which can be explained by maternal allocation of PCBs across a PCB gradient.

Sediment sum PCB concentration values obtained from the eight locations in 2010 correspond with those reported in the literature for this region collected within the last 10 years (Drouillard et al. 2006; Szalinska et al. 2011). The range of sediment sum PCB concentrations observed across the eight locations was not as great as seen in the literature for the Lower Great Lakes region (e.g., lower United States [see Drouillard et al. 2006]). For example, an intensive sediment survey of the Detroit River by Drouillard et al. (2006) described high variability of sum PCB concentrations in regions used in the current study ranging from nondetectable to 1684 ng/g dry weight for upstream Canadian locations (e.g., PI) and ranging from 3 to 1372 ng/g dry weight for downstream American locations (e.g., TC). The limited range we sampled may therefore be explained by the highly localized nature of contaminant "hot spots" in this region (Drouillard et al. 2006; Szalinska et al. 2011) that are not reflected in our sampling or in the spatially integrated exposures experienced by sampled fish. However, the observed sediment sum PCB concentration values for CC, TC, and BI did fall within a range of sum PCB concentrations that have elicited biological effects (Canadian Council 2002). A previous study using brown bullhead originating from two relatively more contaminated habitats also used in the current study, TC and BI, showed differences in life-history traits compared with those found at less contaminated locations, i.e., BR and PI (Farwell et al. 2012). Farwell et al. (2012), however, used individual measures of egg sum PCB concentrations, which exhibited a larger range of PCB concentrations compared with those quantified in sediment samples in the current study. It is also important to note, however, that the rank order of sediment sum PCB concentrations among locations from our study does not correspond with those of egg sum PCB concentrations described by Farwell et al. (2012). This suggests that either our sediment sampling did not reflect a larger-than-expected home range or that site-specific bioavailability of PCBs from sediment differs between compounds altering chemical signatures measured in fish relative to sediments. The differences in PCB ranges, sample type (i.e. tissue vs. sediment), or metabolic rates may explain the lack of observed relationships between PCBs and reproductive life-history traits in the current study.

It was predicted that egg diameter, fecundity, and GSI would decrease with increasing sediment sum PCB concentration. This prediction was not supported because no statistically significant relationships were found between sediment sum PCB concentration corrected for OC and reproductive traits. However, negative relationships were found between sediment sum PCB concentrations not corrected for OC and both egg diameter and GSI, with the latter being nearly significant ($P = 0.07$). This finding suggests that sum PCB concentration is most likely not the contaminant metric driving the significant relationships observed between the sediment sum PCB concentration and the four reproductive traits. Sediment sum PCB concentration not only has a positive relation to other groups of nonpolar organic contaminants in the Lower Great Lakes region, it also has a positive relation to polar inorganic contaminants as well, such as heavy metals (e.g., Szalinska et al. 2011). It is likely that the disparity in our results between the dry and OC weights for sediment sum PCB concentrations is a result of a positive correlation between sediment sum PCB concentration (dry weight) and polar inorganic contaminant concentration, of which bioavailability is not predicted by OC (Weltje 1998). This notion was supported by our PCA, which showed associations between historical metal and dry-weight sum PCB concentrations in the Detroit River area. Metals with the greatest loading values included copper, iron, nickel, and lead, all of which have showed negative effects on reproductive traits and embryo development in fish species (Dave 1985; Munkittrick and Dixon 1988; Dave and Xiu

1991). In addition, we did not account for interactions between contaminant classes on reproductive traits (see Bustness 2006). Observations of smaller egg diameters and GSIs in fish from locations with greater sediment sum PCB concentrations not corrected for OC may have also arisen due to unknown environmental quality characteristics either independently or coinciding with contamination. Differences in food availability across the eight locations were not expected to directly or indirectly impact reproductive trait development because Fulton's condition factor (body size metrics), percent total lipids in eggs, and age did not differ significantly across the eight locations.

It was also predicted that egg carotenoid concentration would decrease with increasing contaminant exposure due to an increase in antioxidant function (Di Giulio et al. 1989; Krinsky 2001; Tyndale et al. 2008). Contrary to this prediction, there was a positive relationship between egg carotenoid concentration and sediment sum PCB concentration not corrected for OC with a 50 % increase in egg carotenoid concentration between the lowest and highest values. Few examples of this positive relationship between egg carotenoid concentration and environmental contaminants have been reported in the literature. However, in laboratory experiments, female American kestrels (*Falco sparverius*) fed a mixture of Aroclor 1254, 1248, and 1260 (1:1:1) also had greater plasma carotenoid concentrations (by ~50 %) and greater carotenoid concentrations in their offspring (by ~50 %) compared with control females (Bortolotti et al. 2003). The concentrations of the Aroclor mixture administered was designed to produce environmentally relevant body and egg burdens and were based on previously collected mammalian and avian muscle and egg samples (Smits and Bortolotti 2001; Fernie et al. 2003).

The positive relationship between egg carotenoid concentration and environmental contamination can be explained by two alternative hypotheses: (1) the reproduction trade-off hypothesis (Bertrand et al. 2006; Monaghan et al. 2009) or (2) the maternal-allocation hypothesis (Blount et al. 2000; Royle et al. 2001). The reproduction trade-off hypothesis predicts that relatively greater amounts of energy invested into reproduction (e.g., increased egg size and/or fecundity) will also cause an increase in oxidative stress in the female (Salmon et al. 2001; Wang et al. 2001). This increase in oxidative stress in turn increases the female's use of her own antioxidants, such as carotenoids (Finkel and Holbrook 2000) and results in a lower amount of carotenoids available to a female to allocate to her eggs compared with a female that invests less into reproduction (e.g., Blount et al. 2002). In the present study, females from less contaminated locations produce larger eggs and therefore may have an overall greater energy investment toward reproduction (GSI) than those from more contaminated environments. If this relationship is explained by the reproduction trade-off hypothesis, it would be predicted that females from less contaminated environments (greater reproduction investment) would have lower carotenoid concentration in both muscle and egg tissues compared with females from more contaminated locations. Alternatively, the maternal-allocation hypothesis states that females will deposit relatively more carotenoids in eggs in uncertain or detrimental environments to enhance offspring survival by way of increased antioxidant and immune function (McGraw et al. 2005; Grether et al. 2008). If the positive relationship between egg carotenoid concentration and environmental contamination is explained by the maternal-allocation hypothesis, it would be predicted that females from less contaminated environments would have lower carotenoid concentrations in their egg tissues and greater carotenoid concentrations in their muscle tissues compared with females from more contaminated locations.

Post hoc analysis was used to investigate the alternative hypotheses as possible mechanisms underlying the positive relationship between egg carotenoid concentration and environmental contamination by comparing carotenoid concentrations in egg and muscle tissues. The maternal-allocation hypothesis was supported with a significant negative relationship between egg and muscle carotenoid concentrations ($r = -0.91$, $P = 0.0018$) with females from less contaminated environments having lower egg carotenoid concentrations and greater muscle carotenoid concentrations compared with females from more contaminated locations (Fig. 3). Little experimental research on the differential maternal allocation of carotenoids in contaminated environments has been performed; however, Sanio et al. (2002) investigated this question in wild populations of barn swallows (*H. rustica*) affected by radiation contamination. They found that females associating with males having manipulated short tails, an indication of radiation exposure, deposited greater amounts of carotenoids in their eggs compared with those associating with "healthy" males. This suggests that females are provisioning offspring expected to have greater radiation exposure with greater amounts of antioxidants to combat negative effects of the radiation. An alternative hypothesis that may explain variation in carotenoids is differences in dietary xanthophyll availability among populations. Although there is no apparent spatial trend among populations, it is possible that differences in food quality could have occurred.

In summary, although this study does not provide evidence of associations between reproductive traits and sediment sum PCB concentrations corrected for OC in brown bullhead, it does provide evidence supporting relationships between reproductive traits and other classes of environmental contaminants whose bioavailability is not predicted by OC, such as heavy metals. A positive relationship was also found between egg carotenoid concentration and sediment sum PCB concentrations not corrected for OC. The results of study suggests that this positive relationship is a result of differential maternal allocation of carotenoids to eggs: Females from less contaminated environments had lower egg carotenoid concentrations and greater muscle carotenoid concentrations compared with females from more contaminated locations. The results of this study identify sublethal effects of environmental contaminants on reproductive traits in female brown bullhead. They also imply that sediment sum PCB concentrations may be used as an indicator of associations between overall environmental contamination and female reproductive traits. This is opposed to lethal tissue sampling, which would be better suited for assessments specifically aimed at PCB and female life-history trait associations. Further research on the ecological implications of between-population differences in the measured reproductive traits is needed to assess whether the observed differences in reproductive traits have an ultimate impact on individual fitness and population-level processes.

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